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Radioprotection of mice following garlic pretreatment

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Summary Freshly prepared aqueous extract of garlic was tested in mice for its possible *in vivo* protective effect against gamma-radiation-induced chromosomal damage. In the same animals, the changes in the sulphydryl content and glutathione S-transferase activity were evaluated. Three doses of garlic extract [125, 250 and 500 mg kg^{-1} body weight (bw)] were administered orally for five consecutive days and the animals were exposed to 0.25, 0.5, 1.0 and 2.0 Gy gamma-radiation 2 h after the final feeding. The results of the bone marrow micronucleus test revealed that pretreatment with garlic extract was effective in reducing gamma-radiation-induced chromosomal damage. Against 0.25 Gy gamma-radiation, a high dose of 500 mg kg^{-1} bw garlic extract was required to significantly reduce the chromosomal damage. All the three doses of garlic extract were effective in exerting a protective effect against 0.5, 1.0 and 2.0 Gy gamma-radiation. However a dose-related effect was observed only against 2.0 Gy. The sulphydryl content and glutathione S-transferase activity registered a significant increase after either pretreatment with garlic extract or irradiation. In the garlic extract pretreated irradiated animals, a significant reduction was observed in the sulphydryl content and glutathione S-transferase activity.

Keywords: garlic; glutathione S-transferase; micronucleus test; radioprotection

Organosulphur compounds which can enhance the activities of detoxication enzymes like glutathione S-transferase, glutathione peroxidase and glutathione reductase are known to be present in garlic (Lin *et al.*, 1994; Liu *et al.*, 1992; Sparnins *et al.*, 1988; Yang *et al.*, 1994). In experimental test systems, garlic extract and its chemical constituents such as allicin, alliin and diallyl sulphide have inhibitory effects on chemical mutagenesis and carcinogenesis (Das *et al.*, 1993; Ip *et al.*, 1992; Knasmüller *et al.*, 1989; Liu *et al.*, 1992; Sparnins *et al.*, 1988). Furthermore, *in vitro* experiments with *Salmonella* tester strains and Chinese hamster ovary cells have shown that garlic can exert antimutagenic effects against gamma-radiation possibly through the scavenging of free radicals (Knasmüller *et al.*, 1989).

In view of the above and the consumption of garlic by humans all over the world, the present investigation was undertaken to evaluate in mice the possible *in vivo* protective effects of freshly prepared garlic extract against chromosomal damage induced by different doses of gamma-radiation ranging from 0.25 to 2.0 Gy. Along with this, the associated changes in sulphydryl (-SH) content and glutathione S-transferase (GST) activity were monitored.

Materials and methods

Animals

All the experiments were carried out with 9–11-week-old male Swiss albino mice weighing 28–32 g. These animals were bred and maintained at $25 \pm 2°C$ on the standard mouse diet (Lipton India Ltd.) and water *ad libitum.*

Chemicals

The chemicals used for the biochemical assays were: 1-chloro-2,4-dinitrobenzene, 5,5′-dithio-bis (2-nitro benzoic acid) and reduced glutathione. These chemicals were obtained from Sigma Chemical Company (St Louis, MO, USA).

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Preparation of garlic extract

An aqueous extract of fresh garlic (purchased from the local market) was prepared using freshly peeled cloves which were homogenised in double distilled water. The homogenate was centrifuged at 3000 r.p.m. for 10 min to remove the particles and the supernatant was used for the experiment. The doses were calculated on the basis of the weight of fresh garlic (mg) used to prepare 1 ml extract.

Pretreatment of mice with garlic extract

Three test doses (125, 250 and 500 mg kg^{-1} bw) of freshly prepared garlic extract were administered by gavage (10 ml kg^{-1} bw) to the experimental animals for 5 consecutive days. The animals were irradiated 2 h after the final feeding. The control animals received the same volume of distilled water.

Irradiation

Whole body irradiation of the experimental animals was carried out in a gamma chamber (source ^{60}Co, 204 TBq, 5500 Ci) obtained from Bhabha Atomic Research Center, Bombay, India. These animals were placed in a well-ventilated polypropylene holder and exposed to gamma-radiation at a dose rate of 0.03 Gy per second.

Micronucleus test

The mouse bone marrow micronucleus test was carried out according to Schmid (1975) for evaluating the chromosomal damage in experimental animals. The animals were sacrificed by cervical dislocation 24 h after irradiation. The bone marrow from both the femurs was flushed in the form of a fine suspension into a centrifuge tube containing fetal calf serum (FCS). This cell suspension was centrifuged at 2000 r.p.m. for 10 min and the supernatant was removed. The pellet was resuspended in a drop of serum before being used for preparing slides. The air-dried slides were stained with May–Grünwald and Giemsa as described by Schmid (1975). For each experimental point 4–5 mice were used and 4000 polychromatic erythrocytes (PCEs) were scored per animal from a single slide to determine the frequency of micronucleated polychromatic erythrocytes (Mn PCEs). All the slides were scored by the same observer.

Biochemical assays

The experimental animals were sacrificed by cervical dislocation 2 and 24 h after irradiation and their livers were excised and perfused thoroughly with ice-cold saline (0.9% sodium chloride). A 10% (w/v) liver homogenate per animal was prepared using buffer containing 0.154 M potassium chloride and 50 mM Tris-HCl (pH 7.4) A portion of the tissue homogenate was used for determining acid soluble sulphydryl (-SH) content, and the remaining portion was centrifuged at 10 000 r.p.m. for 20 min. The resulting supernatant was further centrifuged at 40 000 r.p.m. for 60 min. The supernatant obtained after this centrifugation formed the cytosolic (soluble) fraction which was used for the GST assay. All these steps were carried out at 0–4 °C. The protein content of the cytosolic fraction of each sample was determined using the method of Bradford (1976) with bovine serum albumin (BSA) as standard. The cytosolic GST activity was determined spectrophotometrically as described by Habig *et al.* (1974). The -SH content was estimated using the method of Ellman (1959), as modified by Sparnins *et al.* (1982).

Results

Micronucleus test

The data presented in Figure 1 show the influence of pretreatment with garlic extract (125, 250 and 500 mg kg^{-1} bw) on the frequencies of Mn PCEs induced by 0.25, 0.5, 1.0 and 2.0 Gy gamma-radiation. A significant reduction

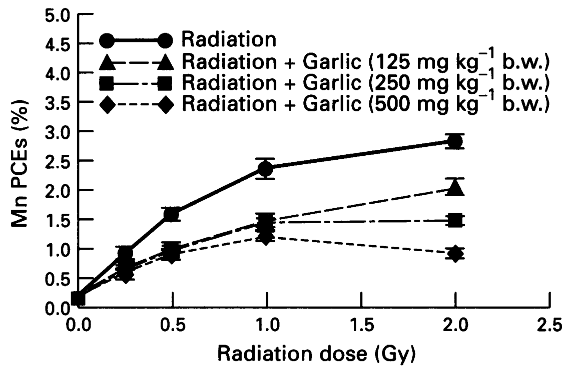

Figure 1 Dose–response for the induction of micronucleated polychromatic erythrocytes (Mn PCEs) after pretreatment with garlic extract.

($P<0.05$) in the frequencies of Mn PCEs induced by 0.25 Gy radiation was observed only after pretreatment with the highest test dose of garlic extract (500 mg kg^{-1} bw). Although all the three doses were effective in significantly reducing ($P<0.01$) the frequencies of the Mn PCEs induced by 0.5 and 1.0 Gy radiation, there was no indication of a dose–response. However, with 2 Gy gamma-radiation, significant dose-related reductions were observed in the frequencies of Mn PCEs after pretreatment with garlic extract.

Biochemical assays

Table I shows the -SH content and GST level in animals sacrificed 2 and 24 h after irradiation. From the 2 h sample, it was evident that garlic pretreatment (500 mg kg^{-1} bw) could significantly increase the -SH content in the irradiated animals. Irradiation alone was also effective in significantly increasing the -SH content in both the samples. Significant reductions in the -SH content were observed in garlic pretreated irradiated animals, irrespective of the sampling time. Unirradiated animals pretreated with garlic (500 mg kg^{-1} bw) and those that received irradiation alone showed significantly increased levels of GST activity in the 2 h sample. The garlic pretreated irradiated animals from the same sample registered a significant reduction in the level of GST activity.

Discussion

From the results of our present study it was evident that pretreatment with garlic extract could reduce the magnitude of *in vivo* chromosomal damage induced by gamma-radiation. This agrees with the findings from *in vitro* studies, which demonstrated the antimutagenic effects of garlic against gamma-radiation in *Salmonella* and Chinese hamster ovary cells (Knasmüller *et al.*, 1989). In order to obtain a significant protective effect against a low dose of 0.25 Gy, it was necessary to administer the highest test dose of 500 mg kg^{-1} bw garlic extract. With increase in radiation dose to 0.5, 1.0 and 2.0 Gy respectively, significant protective effects were observed with all the three doses of garlic extract. The radiation dose–response after pretreatment with garlic (Figure 1) shows a distinct plateau and a decrease in the maximal Mn PCEs level with increase in garlic extract dose. A linear dose–response for the protective effects of garlic extract was observed only against the relatively high dose of 2 Gy gamma-radiation.

In this investigation, biochemical assays were included to monitor the changes in -SH content and GST level. The results show that feeding of garlic extract for 5 consecutive days could lead to an enhancement in the activity of GST, a phase II enzyme involved in the conjugation of electrophiles with glutathione (Wattenberg, 1983). The -SH content also

Table I Sulphydryl (–SH) content and glutathione S-transferase (GST) activity in the livers of irradiated and unirradiated mice pretreated with different doses of garlic and sacrificed 2 and 24 h after irradiation

Group	Radiation dose (Gy)	Garlic dose (mg kg^{-1} bw)	–SH content (umol g^{-1} tissue) 2 h sample	24 h sample	GST activity (umol CDNB–GSH conjugate formed min^{-1} mg^{-1} protein) 2 h sample	24 h sample
A	0	0	6.08 ± 0.05	5.53 ± 0.16	1.10 ± 0.03	1.02 ± 0.08
B	0	125	6.55 ± 0.20*	5.52 ± 0.17	1.19 ± 0.13	1.29 ± 0.13
C	0	250	6.46 ± 0.15*	5.58 ± 0.20	1.04 ± 0.10	1.15 ± 0.02
D	0	500	7.06 ± 0.25**	5.62 ± 0.20	1.30 ± 0.03**	1.14 ± 0.08
E	2	0	7.27 ± 0.17**	6.73 ± 0.12**	1.52 ± 0.09**	0.97 ± 0.04
F	2	125	7.01 ± 0.13	6.06 ± 0.05**	1.22 ± 0.23	0.93 ± 0.05
G	2	250	5.85 ± 0.11**	5.85 ± 0.25**	0.85 ± 0.01**	1.18 ± 0.05
H	2	500	6.78 ± 0.08*	5.82 ± 0.27**	0.83 ± 0.03**	0.94 ± 0.07

*$P<0.05$; **$P<0.01$. (P-values were calculated by comparing A *vs* B; A *vs* C; A *vs* D; A *vs* E; E *vs* F and E *vs* G.)

registered an increase after pretreatment with garlic. Following irradiation of garlic pretreated animals, a significant reduction in GST level was registered. This observation indicates the possible involvement of glutathione in removing the DNA damaging reactive species formed after irradiation. In addition, the possibility of protection through radical scavenging by organosulphur compounds present in garlic cannot be ruled out.

The lowest garlic dose used in our study (125 mg kg^{-1} bw) would approximately correspond to the daily dietary intake of 8 g of fresh garlic by a person weighing 65 kg. This dose significantly reduced the *in vivo* chromosomal damage induced by radiation doses in the range 0.5–2.0 Gy, and it is much lower than the amount consumed in certain regions of China ($\sim$20 g day^{-1}) where a low incidence of gastric cancer has been reported (Amagase and Milner, 1994; Mei *et al*., 1982; You *et al*., 1989). Furthermore, our present work suggests that by increasing the intake of garlic to 500 mg kg^{-1} bw, it may be possible to obtain a moderate level of protection against low doses of radiation which are relevant from the point of environmental exposure.

Since there are many naturally occurring dietary agents showing *in vivo* radioprotective effects (Abraham *et al*., 1993, 1994), it would be of interest to know whether these compounds can act synergistically with garlic in minimising the genetic damage caused by exposure to physical and chemical agents in the human environment. In conclusion, the possible beneficial effects to humans resulting from consumption of garlic will have to be interpreted cautiously because garlic increases the glutathione and GST level (discussed by Lin *et al*., 1994) and thiols are known to enhance the activity of certain chemical carcinogens (Romert and Jenssen, 1987).

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